



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| <p>(21) International Application Number: <b>PCT/US00/05464</b></p> <p>(22) International Filing Date: <b>3 March 2000 (03.03.00)</b></p> <p>(30) Priority Data:<br/> <b>09/263,776</b>                    <b>5 March 1999 (05.03.99)</b>                    <b>US</b> </p> <p>(71) Applicant: AMBI INC. [US/US]; 4 Manhattan Road, Purchase, NY 10577-2197 (US).</p> <p>(72) Inventors: CLIMO, Michael; 1802 Stonecrest Court, Richmond, VA 23236 (US). MURPHY, Ellen; 185 Beach Street, Bronx, NY 10464 (US). ARCHER, Gordon; 1504 Hearthglow Lane, Richmond, VA 23233 (US).</p> <p>(74) Agent: KELBER, Steven, B.; Piper Marbury Rudnick &amp; Wolfe LLP, 1200 Nineteenth Street, NW, Washington, DC 20036-2412 (US).</p>                               |  | <p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b><br/> <i>With international search report.</i><br/> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> </p> |  |
| <p>(54) Title: COMPOSITIONS AND METHODS FOR TREATMENT OF STAPHYLOCOCCAL INFECTION</p> <p>(57) Abstract</p> <p>Co-administration of a lysostaphin or other anti-staphylococcal agent which cleaves cross-links of peptidoglycans of staphylococci cell walls such as lysostaphin and an antibiotic effective against staphylococci due to antibiotic activity mediated by cell-wall activity is effective against staphylococcal infection, even staphylococci that may be resistant to one or other of lysostaphin or the cell-wall active antibiotic. Co-administration simultaneously suppresses the generation of antibiotic-resistant mutant strains. Effective cell-wall active antibiotics include <math>\beta</math>-lactams and glycopeptides.</p> |  |   |  |

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**TITLE OF THE INVENTION**

COMPOSITIONS AND METHODS FOR TREATMENT OF STAPHYLOCOCCAL INFECTION

5

**BACKGROUND OF THE INVENTION****Field of the Invention**

This invention pertains to a method of treating staphylococcal infection in mammals, including humans. The method involves the simultaneous administration of a lysostaphin or other agent which attacks the glycine-containing peptide cross-links of the cell wall 10 peptidoglycan found in staphylococci and an antibiotic, the antibiotic properties of which are mediated by its ability to affect the cell wall of the target staphylococci. This combined administration is effective in treating the staphylococcal infection, and at the same time suppresses the formation of strains resistant to lysostaphin or other peptidoglycan active agent.

15

**BACKGROUND OF THE PRIOR ART**

Lysostaphin is a bacteriocin secreted by a staphylococcal strain isolated and originally named *Staphylococcus staphylolyticus* (now *S. simulans*). The production of lysostaphin is described in U.S. Patent 3,278,378. Lysostaphin is an endopeptidase which cleaves the polyglycine cross-links of the peptidoglycan found in the cell walls of 20 staphylococci. U.S. Patents 3,398,056 and 3,594,284 describe improvements to culture medium and inoculation techniques for the production of lysostaphin.

The gene for lysostaphin from *S. simulans* has been sequenced and cloned, U.S.

Patent 4,931,390. Lysostaphin for use as a laboratory reagent has been produced by  
fermentation of a non-pathogenic recombinant strain of *B. sphaericus*, from which it is  
readily purified. The cloning and sequencing of the lysostaphin gene permits the isolation  
5 of variant enzymes that have properties similar to or different from those of wild type  
lysostaphin. One such altered enzyme, bearing a single amino acid change, has been  
characterized and shown to have potent anti-staphylococcal activity both *in vitro* and in an  
animal infection model. U.S. Patent Application Serial No. 09/120,030, filed July 21, 1998  
and incorporated herein by reference. Other lysostaphin analogues, including naturally  
10 occurring enzymes of this type have been established as potent agents capable of addressing  
difficult to treat bacterial diseases caused by staphylococcal infection. Other peptidases  
with related activity are known. Thus *lasA* protease and achromopeptidase, reported in  
Kessler et al., J. Biol. Chem. 268:7503-08 (1993) and Li et al., J. Biochem. 122:772-  
778(1997), respectively, have anti-staphylococcal activity based on their digestion of  
15 glycine-containing cross-links in the peptidoglycan cell wall component. These agents may  
be used in this invention in place of lysostaphin.

The development of lysostaphin as an effective antibiotic to treat staphylococcal  
infection has been plagued, however, by a problem that is universal for antibiotic  
administration - the increasing development of antibiotic-resistant strains of mutant  
20 staphylococci. Already, a wide variety of staphylococcal infections resistant to various  
antibiotics that were previously the treatment of choice, including methicillin (methicillin  
resistant *S. aureus* are referred to as MRSA) and vancomycin-resistant strains (referred to  
as VISA) have been identified. Resistance to a wide variety of other antibiotics, not

exhibited by sensitive staphylococci, has been noted as well. MRSA, as well as strains resistant to other antibiotics, are discussed at length in Stranden, et al., J. Bacteriology 179(1):9-16 (1997). Further difficulties are encountered in that MRSA tend to accumulate a variety of other resistances as well. Multiresistant MRSA are typically treated with 5 vancomycin, The Staphylococci In Human Diseases, 158-174 (Grossley, et al., editors 1997). Vancomycin itself may be toxic. Additionally, vancomycin resistance has recently been detected in staphylococci infections.

The problem posed by the continuing development of antibiotic-resistant infectious agents, such as staphylococci, is more than the difficulty involved in treating any individual 10 patient. Popular press, as well as scientific journals, have noted the alarming increase in the generation of resistant strains, due in part to indiscriminate use or over-use of antibiotics. Each time an individual is treated with an antibiotic, whether needlessly or reasonably, the chance that a strain resistant to that particular treatment will arise is increased. Resistant strains of staphylococci have become endemic in many hospitals and 15 pose a life-threatening danger to patients already debilitated by other ailments who become infected after admission to those hospitals.

Numerous articles have noted the development of resistance to either lysostaphin or  $\beta$ -lactams, such as methicillin, and the relationship there between. Thus, DeHart, et al., Applied Environmental Microbiology 61, 1475-1479 (1995) noted the development of 20 mutant *S. aureus* recombinant cells that were resistant to lysostaphin, but susceptible to methicillin. Similar phenomenon are reported by Zygmunt, et al., Can. J. Microbio. 13,845-852 (1966), Polak, et al., Diagn. Microbiol. Infet. Dis. 17:265-270 (1993) and Dickson, et al., Yale J. Bio. Med. 41:62-67 (1968). Each of these references, as well as

later reports such as Ehrent, J. Bacteriology, 179:7573-7576 (1997) note that staphylococci that develop resistance to lysostaphin, either spontaneously or through induced recombination, become susceptible to methicillin treatment, and vice-versa. In all of these references, the uniform suggestion is to follow a course of administration of lysostaphin, even a short one, with administration of methicillin.

5 U.S. Patent 5,760,026, commonly assigned herewith, employs a specific method for treating mastitis, by intramammary infusion of lysostaphin. The patent reports, Table ID and elsewhere, that a synergistic result is predicted when combining lysostaphin and a  $\beta$ -lactam to treat mastitis, based on an *in vitro* assay. The bovine mastitis model is not predictive of *in vivo* administration of antibiotics, and the synergistic effects reported in 10 U.S. Patent 5,760,026 have not been substantiated in an environment or model that would be reflective of *in vivo* administration to a mammal such as a human.

Those of skill in the art will be aware that there are a wide variety of staphylococcal strains. Many are resistant to conventional antibiotics, unlike sensitive strains. *S. aureus* 15 strains are recognized as highly virulent and the most common single cause of serious systemic infections. Coagulase-negative staphylococcal species, although generally less invasive than *S. aureus*, are now responsible for a significant incidence of infections; particularly among debilitated or immunocompromised patients. As an example of such infection, one may point to endocarditis consequent to heart valve replacement. This is but 20 one of a variety of intractable staphylococcal infections which are increasing due to the widespread use of antibiotics.

Accordingly, it remains an object of those of ordinary skill in the art to develop a method whereby even resistant staphylococcal infections in mammals, including humans,

may be effectively treated by the administration of antibiotics. Desirably, this method is developed so as suppress the formation of strains resistant to the antibiotics used.

## SUMMARY OF THE INVENTION

The above goals, and others made clear by the discussions set forth below, are  
5 achieved by the simultaneous administration of an anti-staphylococcal agent, such as lysostaphin or other agent whose activity is mediated by cleavage of glycine-containing cross-links in the staphylococcal cell wall peptidoglycan and an antibiotic or antimicrobial agent whose activity is mediated by its ability to affect the cell wall of staphylococci.  
These cell-wall active agents include  $\beta$ -lactams and glycopeptides. Preferably, the cell-  
10 wall active antibiotic is a  $\beta$ -lactam.

There is no evidence of any synergistic effect achieved through the simultaneous administration of an anti-staphylococcal agent whose activity is mediated by cleavage of glycine-containing cross-links and a cell-wall active antibiotic in a model, *in vitro* or *in vivo*, that is predictive of benefit for *in vivo* administration of antibiotics in a mammal.

15 Indeed, those of ordinary skill in the art will recognize that for resistant staphylococci, such as MRSA, the administration of methicillin is not therapeutically effective in any amount. Surprisingly, Applicants have discovered that the combined administration of an anti-staphylococcal agent whose activity is mediated by cleavage of glycine-containing cross-links such as lysostaphin and the cell-wall active antibiotic not only effectively treats the  
20 infection, but suppresses the formation of staphylococci having resistance to the anti-staphylococcal agent whose activity is mediated by cleavage of glycine-containing cross-links.

- While Applicants do not wish to be bound by this explanation, it appears that the spontaneous mutation commonly effective in conferring lysostaphin resistance in staphylococci renders the same highly susceptible to a cell-wall active antibiotic, such as methicillin. This is true even where the organism starts out as methicillin resistant.
- 5      Simultaneous administration of both appears to be uniformly effective in simultaneously eradicating the infection and suppressing the generation of new resistant strains. Specifically, anti-staphylococcal agents like lysostaphin cleave glycine-containing cross-links. The mutation conferring resistance to this attack renders previously resistant strains sensitive to cell wall active antibiotics.

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#### DETAILED DESCRIPTION OF THE INVENTION

This invention involves the administration of a pharmaceutical composition effective in the treatment of staphylococcal infection, which composition comprises at least two active agents, one an agent like lysostaphin which cleaves the glycine-containing cross-links of the cell wall peptidoglycans of staphylococci, the other a cell-wall active antibiotic. By lysostaphin it is intended to refer herein to any enzyme, including lysostaphin wild type, a mutant or variant, or any recombinant or related enzyme that retains proteolytic activity against glycine-containing cross-links in the cell wall peptidoglycan of staphylococci. Variants may be generated by post-translational processing of the protein(either by enzymes present in a producer strain or by means of enzymes or reagents introduced at any stage of the process) or by mutation of the structural gene. Mutations may include site-deletion, insertion, domain removal and replacement mutations. They may be recombinantly expressed, or otherwise. Other anti-staphylococcal active agents

acting by cleavage of the glycine-containing peptidoglycan cross-links include *lasA* protease and achromopeptidase. Such anti-staphylococcal agents which affect the peptidoglycan cross-links are embraced by the invention, but exemplified herein by reference to lysostaphin.

5 Cell-wall active antibiotics include  $\beta$ -lactams and glycopeptides.  $\beta$ -lactams are preferred. Suitable  $\beta$ -lactams include, but are not limited to, penicillins, such as penicillin, nafcillin, oxacillin, methicillin, amoxicillin and cloxacillin. Other  $\beta$ -lactams include cephalosporins and carbapenems. Representative cephalosporins include cephalothin, cefazolin, cefamandole, ceftazidime and others. Suitable carbapenems include imipenem  
10 and meropenem.

Suitable glycopeptides include vancomycin, teicoplanin and ramoplanin.

These two agents can be combined with further agents, adjuvants and the like, but are effectively administered in a pharmaceutically acceptable carrier. Administration is typically systemic, and may be intravenous (IV), intramuscular (IM), subcutaneous (SC),  
15 intraperitoneal (IP), intrathecal or topical. No synergistic effect of combining lysostaphin and a  $\beta$ -lactam or glycopeptide or cell-wall active antibiotic has been noted in a model predictive of *in vivo* mammalian administration. Accordingly, each agent of the effective combination must be administered in a therapeutically effective amount. It is to be noted, in this regard, that the amount to be administered is that which is therapeutically effective  
20 when the lysostaphin and cell-wall active agent are administered together. Those of skill in the art will of course recognize that there is no therapeutically effective amount for, e.g., methicillin if the staphylococcal infection is an MRSA infection. Nonetheless, administration of therapeutic amounts of methicillin as determined against non-MRSA,

combined with an amount of lysostaphin effective against staphylococci that are not lysostaphin-resistant will effectively treat staphylococcal infections even where the infection is resistant to one or other antibiotic. Accordingly, applicants have referred herein to "therapeutically effective amounts" to mean amounts effective to therapeutically treat 5 sensitive *S. aureus* infection. This simultaneous administration, as opposed to sequential administration typified by the prior art, also surprisingly results in the suppression of strains resistant against either antibiotic, or their combination.

Any of a wide variety of pharmaceutically acceptable carriers and diluents, typically buffered, may be used. Appropriate pharmaceutical carriers are known to those of skill in 10 the art. The formulations of this invention comprise a therapeutic amount of lysostaphin and a therapeutic amount of a cell-wall active antibiotic, such that when co-administered, the staphylococcal infection, either *S. aureus* or coagulase negative staphylococci, is treated, while the generation of resistant strains is suppressed. Other active agents that do not interfere with the activity of the two antibiotics may be co-administered.

15 Therapeutic values will range substantially given the nature of the staphylococcal infection, the individual, and the antibiotic being used in conjunction with lysostaphin. Representative values for anti-staphylococcal active agents such as lysostaphin, range from approximately 15-150 mg/kg body weight/day for human administration, with a preferred range of 25-100 mg/kg/day. Values for  $\beta$ -lactams based on nafcillin range from 50-250 20 mg/kg/day, with a preferred range of 100-200 mg/kg/day and glycopeptides like vancomycin are administered over a range of 10-75 mg/kg/day, with a preferred range of 15-50 mg/kg/day.

The administration course is not substantially different from that currently administered in single antibiotic treatments, and can range from 7-28 days, although typically, courses of 7-21 days are employed, and effective in treating a wide variety of staphylococcal infections.

5

## EXAMPLES

To compare the development of resistant strains, growth curves for three methicillin resistant staphylococcal strains were obtained for *in vitro* growth in Mueller Hinton Broth.

Growth curves were completed in Mueller Hinton Broth (50 ml) in glass erlenmeyer flasks. Flasks were inoculated with 100  $\mu$ l of an overnight growth adjusted to 0.5 Macfarland to yield a starting concentration of approximately  $10^5$  -  $10^6$  CFU/ml. Growth curves were done in the presence of lysostaphin, lysostaphin and oxacillin (1  $\mu$ g/ml) or no antibiotics (controls). Absorbance at OD 600 was recorded at 0, 2, 4, 6 and 24 hours. At 24 hours flasks were plated on MHA, MHA with lysostaphin (6  $\mu$ g/ml) and MHA with oxacillin (6  $\mu$ g/ml) in order to screen for resistant mutants. Three methicillin resistant 15 *Staphylococcus aureus* strains were tested: 272855, 450M and Mu3.

Growth following 24 hour incubation with lysostaphin (0.0625  $\mu$ g/ml), lysostaphin (0.0625  $\mu$ g/ml) and oxacillin (1  $\mu$ g/ml), and no antibiotics, was recorded.

The data generated led to the following conclusions:

1. The addition of oxacillin to lysostaphin led to significant suppression of growth 20 for all three strains.
2. The presence of oxacillin suppressed the expression of lysostaphin resistance among all three strains.

In order to demonstrate the effectiveness of the claimed invention, certain experiments were conducted. Checkerboard susceptibility testing was conducted to determine whether simultaneous administration of lysostaphin and oxacillin (a  $\beta$ -lactam) would be effective in suppressing the development of resistance. Oxacillin concentrations varied between 0.0156  $\mu\text{g}/\text{ml}$  and 1  $\mu\text{g}/\text{ml}$ . Lysostaphin concentrations varied between 0.00048 and 0.9  $\mu\text{g}/\text{ml}$ . Four strains were tested for evidence of synergy between lysostaphin and oxacillin; 27619, Col, 27227 and VA348. There was no evidence of synergy or antagonism over the concentration range tested. The MIC of lysostaphin was unchanged in the presence of oxacillin in concentrations up to 1  $\mu\text{g}/\text{ml}$  for all strains tested.

5 The overnight growth of strains in the presence of lysostaphin and oxacillin was examined. Four strains were grown overnight in drug free media (MHB), MHB with 0.1  $\mu\text{g}/\text{ml}$  of lysostaphin, MHB with lysostaphin 0.1  $\mu\text{g}/\text{ml}$  and oxacillin 1  $\mu\text{g}/\text{ml}$ , and MHB with oxacillin 1  $\mu\text{g}/\text{ml}$ . The four strains tested included 450M, Col, and their lysostaphin resistant mutants 450 M lyso and Col lyso. The results are reflected in Table 1.

10

15

TABLE 1

| Isolates  | Growth in the presence of |   |                                   |                 |
|-----------|---------------------------|---|-----------------------------------|-----------------|
|           | MHB                       | lysostaphin 0.1 $\mu\text{g}/\text{ml}$ | oxacillin $\mu\text{g}/\text{ml}$ | Lyo + oxacillin |
| 450M      | +                         | +                                       | +                                 | -               |
| 450M lyso | +                         | +                                       | -                                 | -               |
| Col       | +                         | +                                       | +                                 | -               |
| Col lyso  | +                         | +                                       | -                                 | -               |

20

The same unpredicted result has been demonstrated through *in vivo* experiments based on the widely accepted rabbit model of aortic valve endocarditis, predictive of *in vivo*

administration to humans. When administered to staphylococcal infected rabbits at low doses (1 mg/kg bid, as compared with a minimum value of 5 mg/kg tid for sterilization) lysostaphin, as representative of anti-staphylococcal agents acting by cleavage of the glycine-containing cross-links, resulted in recovery of a number of resistant colonies, with 5 high counts in vegetations and kidneys, while the same dosage together with nafcillin (a  $\beta$ -lactam) gave sterile kidneys, some sterile vegetations, and no resistant strains recovered. The simultaneous treatment of staphylococcal infection with suppression of resistant strain formation is an exciting and widely useful invention nowhere predicted in the art. This invention offers the possibility of treating staphylococcal infections while suppressing the 10 generation of strains resistant to any or all active agents administered.

The inventive compositions and methods of this application have been disclosed generically, and by reference to specific example, examples are not intended to be limiting unless so indicated, and variations will occur to those of ordinary skill in the art without the exercise of inventive faculty. In particular, variations in the identity of the cell-wall active 15 antibiotic to be co-administered with an anti-staphylococcal agent acting by cleavage of the glycine-containing cross-links , as well as various recombinant and mutant variants thereof, carriers and concentrations will occur to those of skill in the art without the exercise of inventive faculty, and remain within the scope of the invention, unless specifically excluded by the claims set forth below.

**WHAT IS CLAIMED IS:**

1. A method of treating a staphylococcal infection in a mammal in need of same, comprising administering an antimicrobial agent thereto while suppressing the formation of staphylococci mutant strains resistant to said antimicrobial agent, said method comprising  
5 simultaneously administering an amount of an anti-staphylococcal agent whose activity is mediated by cleavage of the glycine-containing cross-links of peptidoglycans of the cell wall of staphylococci (peptidoglycan active agent) and an amount of another antibiotic effective against sensitive staphylococci, wherein said antibiotic activity is mediated by cell-wall activity (cell wall active antibiotic), whereby the formation of staphylococci mutant strains  
10 resistant to said peptidoglycan active agent is suppressed, wherein said amount of peptidoglycan active agent and said amount of said cell-wall active antibiotic are each individually sufficient to be therapeutically effective against sensitive staphylococci, when co-administered.
2. The method of Claim 1, wherein said peptidoglycan active agent is lysostaphin
- 15 3. The method of Claim 1, wherein said administration is achieved through any one or more of intravenous (IV), intramuscular (IM), subcutaneous (SC), intraperitoneal (IP), intrathecal or topical administration.
4. The method of Claim 3, wherein said administration is SC, IP, intrathecal or topical.
- 20 5. The method of Claim 3, wherein said administration is either IV or IM.
6. The method of Claim 1, wherein said cell wall active antibiotic is a  $\beta$ -lactam or a glycopeptide.
7. The method of Claim 6, wherein said cell-wall active antibiotic is a  $\beta$ -lactam.

8. The method of Claim 7, wherein said  $\beta$ -lactam is selected from the group consisting of a penicillin, a cephalosporin and a carbapenem.
9. The method of Claim 8, wherein said  $\beta$ -lactam is a penicillin.
10. The method of Claim 1, wherein said staphylococcal infection is mediated by at least one *S. aureus* microorganism.  
5
11. The method of Claim 1, wherein said staphylococcal infection is mediated by at least one coagulase-negative staphylococcal microorganism.
12. A composition effective in the treatment of staphylococcal infection in a mammal, comprising, as active agents, an anti-staphylococcal agent whose activity is mediated by cleavage of the glycine-containing cross-links of peptidoglycans of the cell wall of staphylococci (a peptidoglycan active agent) and an amount of another antibiotic effective against sensitive staphylococci, wherein said antibiotic activity is mediated by cell wall activity (a cell wall active antibiotic), further comprising a pharmaceutically acceptable carrier, wherein each of said peptidoglycan active agent and cell-wall active antibiotic are individually present in amounts which are therapeutically effective in treating a sensitive staphylococcal infection.  
10
13. The composition of Claim 12, wherein said anti-staphylococcal peptidoglycan active agent is lysostaphin.  
15
14. The composition of Claim 12, wherein said cell-wall active antibiotic is a  $\beta$ -lactam or a glycopeptide.  
20
15. The composition of Claim 14, wherein said cell-wall active antibiotic is a  $\beta$ -lactam.

## INTERNATIONAL SEARCH REPORT

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| International application No.<br>PCT/US00/05464 |
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**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : A61 K 31/00, 31/43, 31/48, 31/545  
 US CL : 514/02, 06, 11, 12, 192, 200

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/02, 06, 11, 12, 192, 200

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|-----------|---|-----------------------|
| X         | US 5,760,026 A (BLACKBURN et al.) 02 June 1998, see claim 4 and Table ID.   | 1-17                  |
| X         | US 5,858,962 A (BLACKBURN et al.) 12 January 1999, see claims 1,11, Table ID  | 1-17                  |
| X         | POLAK et al. In vitro activity of recombinant lysostaphin-antibiotic combinations toward methicillin-resistant staphylococcus areas. Diagn. Microbiol. Infect. Dis., 1993, Vol. 17, pages 265-270, see abstract and tables 3,4. | 1-17                  |

Further documents are listed in the continuation of Box C.  See patent family annex.

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